Program/Abstract #510
How somitic cells migrate into the axolotl limb bud and vertebrate appendicular muscle evolution
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Skeletal muscles of the vertebrate trunk and limbs are derived from somites. There are different mechanisms proposed for how somitic cells reach the limb bud. It is presumed that the ancestral mode involves an epithelial extension of the somites, as represented by chondrichthyans and some teleost fishes. The derived mode, where individual muscle progenitor cells migrate from the somites into the fin/limb bud, has been reported in chickens, mice and zebrafish. This finding in zebrafish led to the conclusion that the genetic mechanism for tetrapod limb muscle development evolved prior to the radiation of sarcopterygians. However, in studies of non-avian reptiles, the ancestral mode of epithelial extensions of the somites has been reported. Amphibians represent a key group to further unravel the evolutionary history of limb muscle development. Previous studies in amphibians have been complicated due to the delayed development of limb buds relative to the somites. We use transgenic fate mapping techniques in the axolotl to analyze the mode of limb muscle formation. Furthermore, we characterize the somitic cells contributing to the limb by examining the expression of migrating muscle precursor markers, including lbx1 and mox2. The results obtained from the axolotl will provide further insights into the evolution of appendicular muscle development.

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Program/Abstract #511
The embryonic origin of the axolotl skull (*Ambystoma mexicanum*)
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The vertebrate skull is derived from two different embryonic cell populations, neural crest and mesoderm. The fates of these cell populations and their respective contributions to cranial cartilages and bones have been studied in great detail using the quail-chick chimeric system. Enabled by technical advances, such studies have been extended recently to vertebrates other than birds, such as mouse and the African clawed frog, *Xenopus laevis*. This work has facilitated a comparative consideration of the embryonic origin of the vertebrate skull based on experimental data. They are important topics that remain to be elucidated, such as the extent of interspecific differences in skull segmentation and variability in the embryonic origin of specific bones, e.g., frontal and parietal. We are trying to address these issues by extending the fate-mapping approach to a urodele, the Mexican axolotl (*Ambystoma mexicanum*). Unlike *Xenopus*, axolotls are obligately neotenic, and thus do not metamorphose. The skull is initially cartilaginous, but develops bones in later stages of development. We performed long-term fate mapping experiments of cranial neural crest using GFP-transgenic axolotls, to assess its contributions to the skull. These experiments may reveal patterns of derivation unique to anurans and salamanders, or even to amphibians. In a broader context, new data obtained will enhance our knowledge of constrained and variable features of skull derivation, and of the evolution of the vertebrate head in general.

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Program/Abstract #512
Major shifts in the evolution of somitogenesis: The reptile *Anolis carolinensis* represents a fourth type of segmentation clock among vertebrates
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In vertebrates, while the hairy-enhancer of split (hes) genes are the core oscillators of somitogenesis, the mechanism by which hes genes drive Notch receptor activation has evolved divergently. In teleosts, Notch is cyclically activated by deltaC ligand, but in mammals and birds, delta ligands do not display oscillatory expression and Notch is cyclically inhibited by Lunatic fringe. In mammals, Notch is further inhibited by Dll3, a non-cycling, divergent ortholog of deltaC, which is absent from avian genomes. To better understand the evolution of somitogenesis, we identified key regulators of somitogenesis in the *Anolis carolinensis* lizard model, which is the first sequenced non-avian reptile, and found a surprising divergence from other vertebrates. In *Anolis*, lunatic fringe is not a cycling gene; it is expressed in somites but absent from the presomitic mesoderm (PSM). Intriguingly, unlike any other vertebrate, the *Anolis* dll1 orthologue displayed cycling expression in the PSM. The dll2 *Anolis* gene, a divergent ortholog of X-Delta-2, deltaC, and Dll3, is dynamic in expression level. Other components of the segmentation clock are conserved with other vertebrates, including hes7 cycling and the expression of tbx6, fgf8, and mesp2. No components of the Wnt or FGF pathway, which are oscillatory in mammals, were found to cycle in *Anolis*. These findings suggest there have been at least four major switches in the evolution of somitogenesis. Further molecular analysis of unstudied groups, such as chelonian and crocodilian reptiles and caudate amphibians, will help address this hypothesis. Acknowledgments: The Broad Institute for pre-publication release of Anolis genomic sequence; ASU School of Life Sciences.

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Program/Abstract #513
Morphology and regression of the dental lamina
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Tooth development initiates with the formation of an oral epithelial thickening. This thickening grows deeper into the mesenchyme and forms the dental lamina. Here, we focus on differences in the development of the dental lamina among mono- (chameleon) and polyphyodont (python, pit-viper, gecko) reptiles and mono- (mouse) and diphyodont (pig) mammals. We aim to compare the timing of dental lamina development, with specific focus on the initiation of replacement teeth, comparing the structure and cell dynamics of the dental lamina in different species. Dental lamina growth was angled in the lingual direction for all investigated species. The formation of tooth germs in the monophyodont species was initiated in close proximity to the oral epithelium, however chameleon tooth germs in contrast to mouse developed as asymmetrical structures with a large cervical loop on the lingual side. The replacement lamina formed in diphyodont species as the primary dentition reached the late bell stage. As the replacement generation

was initiated in the diphyodont pig, the dental lamina between the oral epithelium and the primary tooth became disconnected and fragmented into several pieces. The degradation of the pig lamina occurred during mid-gestation and injection of DiI into the dental lamina revealed high migratory potential of the lamina cells towards the mesenchyme. The dental lamina of all examined reptiles was not interrupted and kept a compact morphology, connecting the individual tooth generations, including fang teeth, during prehatching and posthatching stages. From such observations it is possible that the degradation of the dental lamina plays a role in restricting the number of tooth generations. MB and OZ are supported by GAASCR (grant KJB601110910) and IRP IPAG No. AVOZ 5045015, AST is supported by an International Joint grant from the Royal Society (JP080875).

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Program/Abstract #514
Filling in the gaps: First look at neural crest migration in a non-Avian reptile
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The morphological architecture of all species results from a genomic-environmental interaction, from which the best fit is selected for a particular environmental niche. In vertebrates, the skull is the most architecturally diverse and embryologically complex anatomical feature of an organism and can tell us much about its life history. Neural Crest Cells (NCCs), a population of cells arising from the dorsal neural tube, migrate and provide the progenitor cells for most of the craniofacial tissues of vertebrates. Thus, playing a key role in cranial development, their highly conserved migratory patterns are of importance for comparative and medical study. Herein, we provide the first analyses of the patterns of neural crest cell migration and development in a squamate (i.e., phylogenetic group comprising lizards and snakes), the Veiled Chameleon (Reptilia: Squamata: Chamaeleonidae). Due to the phylogenetic placement of Reptilia as the sister group to Mammalia, using a squamate as an outgroup to study craniofacial development and evolution of NCCs provides the advantage of maintaining a more 'typical' diapsid cranial architecture (though still missing the lower temporal bar present in Sphenodontia) and thus lacking the extremely derived avian cranial architecture. Developmentally, this species provides a more suitable system for studying early embryonic development within squamata and also maintains a unique array of cranial, trunk and appendicular skeletal morphologies for studying functional and ecological adaptations.

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Program/Abstract #516
Uncovering the ancestral role of FGF signaling in neural development
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FGF signaling plays prominent roles in early neural development including in A-P patterning and neural induction. The role of FGF signaling during deuterostome neural induction, however, remains controversial and varies in the organism studied. In *Ciona* and chick, FGF is a key instructive signal for neural induction, whereas in *Xenopus*, it facilitates induction by inhibition of BMP signaling (neural default) and is necessary to maintain neural progenitor proliferation. It has proven difficult to tease out a role for FGF in neural induction that is independent of its role to reinforce the default pathway by either inhibiting BMP signaling or inducing mesodermal tissue. To help clarify these complexities, we are studying the role of FGF in the neural development of Saccoglossus kowalevskii, an organism in which neural induction is not dependent on BMP/Chordin antagonism. Furthermore, Saccoglossus is ideally situated at the base of the deuterostome phyla to elucidate the ancestral role of FGF signaling in neural development. While the long term goal of this work is to determine how the specification of a diffusely organized neuronal cell population is achieved independent of the conserved BMP/Chordin antagonism, here, we analyze the role of the FGF pathway in neural development.

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Program/Abstract #517
Vertebrate kidney innovation by ponzr1
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The homeobox (hox) and paired box (pax) master regulatory gene families provide a foundation for animal body plan and organ structure. Master regulatory genes establish core pathways that control organogenesis. Despite these conserved genes, organs are expanded and modified in response to evolutionary pressures. How organ variation is encoded in the genome remains an open question. We molecularly and functionally characterized one member of an evolutionarily dynamic gene family, plac8 onzin related protein 1 (ponzr1), in the zebrafish. Genetically, ponzr1 functions downstream of pax2a and forms a feedback loop that modifies pax2a expression. Morpholino knockdown reveals ectopic midline expression of pax2a and wt1a at 24 hour post-fertilization (hpf). At 72hpf, ponzr1 knockdown results in a modified kidney with loss of the glomerulus and disrupted podocytes. Despite glomerular loss, the resulting kidney is a functioning structure reminiscent of the kidney found in aglomerular fish. Therefore, we propose a new model of kidney development wherein pax2a signals for kidney differentiation in the pronephric ducts and tubules, while ponzr1 serves as a switch to signal for a more complex kidney that filters with an integrated glomerulus. Examining a second organ system involved in osmotic homeostasis, we find that the wt1b -expressing pharyngeal arches, which will develop into the gills, do not form in ponzr1 morphants. We functionally demonstrate that ponzr1 can act as a transcription factor or cofactor. Together this work provides experimental evidence of an additional mechanism that incorporates evolutionarily dynamic, lineage-specific gene families into master regulatory gene networks to create functional organ diversity.

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